IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

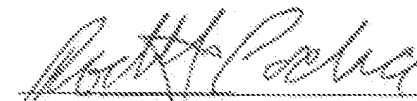|			
Applicants:	Daniel C. Sigg, et al.	Examiner:	Christopher Koharski
Serial No.	10/647,522	Group Art Unit:	3763
Filing Date:	August 25, 2003	Docket No.:	P0011031.00
Title:	ELECTROPORTATION CATHETER WITH SENSING CAPABILITIES		

DECLARATION UNDER 37 C.F.R. § 1.131 ANTEDATING A REFERENCE

I hereby declare the following:

1)   I am currently and correctly named as an inventor in the pending patent application entitled "ELECTROPORTATION CATHETER WITH SENSING CAPABILITIES", U.S. patent application serial number 10/647,522.

2)   The invention disclosed within the above-referenced patent application was conceived of by me and the other named inventors before March 14, 2002.

3)   An Invention Disclosure Form was completed that described the invention and was submitted to the Medtronic, Inc. legal department for consideration before March 14, 2002 (a redacted copy of said form is attached hereto).

5)   I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: May 16, 2007

_____
Rodolfo Padua

DISCLOSURE FILE

Docket No.: P11031.00

Status: O   Division: LB011
Substatus: REV   Attorney: MCS
SubDivision: LB011

Title: DEVICE AND METHOD FOR ACUTE DELIVERY OF MACROMOLECULES TO THE HEART VIA ELECTROPORATION

Inventors: Sigg, Daniel C.
Cross, Daisy
Casas-Bejar, Jesus Wilfredo
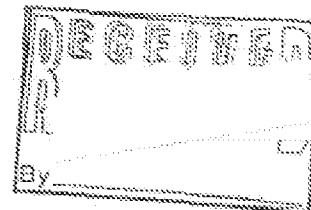Padua, Rodolfo

Submitted Da

Priority:

Approved to File:

Related ID:

Outside Counsel:

Licensee: License File No.:

Other Information:

Minutes:

Medtronic

INVENTION DISCLOSURE FORM

WARNING: Due to the confidential nature of this document, save it as a password protected document. Do not send this document through GroupWise.

This is a WORD Template form. Press enter or tab to move to each field. Please fill out this form as completely as possible. If the allotted space is not sufficient, use a separate sheet. Have your manager sign the form and forward it to the Patent Section of the Law Department, LC340. Please attach any drawings and technical descriptions that are available and assemble copies of the background articles, books, advertisements, etc. for use by your patent attorney.

1.

Inventor(s) Full Name(s)	Employee Number	Mail Stop	Home Address (Include Zip Code)
Daniel C Sigg	65922	B252	1485 Hoyt Ave W, Saint Paul, MN 55108
Daisy P Cross	84118	LT220	2548 Fillmore St. NE, Minneapolis, MN 55418
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Rudy Partus	33848	LT 220	7314 3rd Ave S, Richfield, MN 55423

2.   Title of Invention: **Device and Method for acute delivery of macromolecules to the heart via electroporation**

3.   Summary of the Invention: **This device describes a catheter system that allows acute fluid delivery to heart to multiple sites. It incorporates a steerable catheter which contains an extendable and retractable fluid delivery catheter. The hollow stylet may be removable or contained within the delivery catheter. The delivery catheter and the fluid delivery cather have sensing capabilities to assure contact with the myocardium during injection into the myocardium. This catheter is able to electroporate the cell surrounding the fluid delivery catheter tip which could be a helix or a different needle design to facilitate the transport of macromolecules from the outside of the cells into the cells, once they were injected into the tissue.**
Electroporation is a method used to facilitate the transfer of relatively large molecules, such as plasmid DNA, into cells. This method of transfection involves the application of a high intensity electric field across cells for a short time, during which transient transmembrane pores may be formed. Plasmid DNA can gain access into the cell through these pores and may be incorporated into the host cell's genome. Because cardiac tissue is electrically active, it may therefore be especially receptive to electroporation-mediated gene transfer (1). It has already been reported that electroporation can occur during defibrillation (2,3). In the presence of exogenous genes, it is our hypothesis that this phenomenon can be exploited and used towards a therapeutic advantage. The combination of successful, controlled electroporation-mediated gene transfer with Medtronic's strength in defibrillation and bioelectric technology could result in a promising line of therapy for cardiac-related abnormalities.

References
1.   Harrison RL, Byrne BJ, and Tung L. Electroporation-mediated gene transfer in cardiac tissue. FEBS Letters 435: 1-5 (1998).
2.   Tovar O and Tung L. Electroporation and recovery of cardiac cell membrane with rectangular voltage pulses. Am. J. Physiol. 263: H1128-H1136 (1992).
3.   Krassowska W. Effects of electroporation on transmembrane potential induced by defibrillation shocks. PACE 18 (Pt 1): 1644-1660 (1995).

4.   How have others addressed this problem (List and attach any patents, books, articles, devices, Medtronic or competitor's products, or other background materials you used or which may be prior art)? Patent application P10537

5.   The invention is described on pa   ok No. 10191 (Please attach copy).

Also: DC's notebook 10893 p.   s

6.   When was a device built which included the ...

Who built it? John Sommer   Where is it? Daniel Sigg's ...

Who has supporting documents? John Sommer, Daniel Sigg

Who witnessed tests? _Daisy Cross, Daniel Sigg, James Coles_   When and where? _U of MN, Visible Heart Laboratory,_
_5/08/02, 5/17/02_

7. Discuss the problem which the invention is designed to solve, referring to any prior devices of a similar nature with which you may be familiar.   This device is designed for acute fluid delivery to the heart to multiple sites. A mapping catheter aids location of appropriate sites and subsequent injection. Injection is safely performed as a electrical signal is obtained from the needle tip of the fluid delivery catheter component when it is in contact with the myocardial tissue. As gene delivery is often associated with poor transfection (effectiveness), this device provides an electrical shock to electroporate the cardiac cells to transiently make the cell membranes permeable to macromolecules.

8. State the advantages of the invention over presently known devices, systems or processes.   **An electroporation/gene delivery approach could elimininate the need for a gene delivery vector such as a virus which by itself has potential problems (immune response, contamination, expensive and labor intensive production and overall high cost). Plasmids alone are/may be better than virus; and electroporation enhances plasmid delivery. Obviously, the electrode to delivery the electroporation shock is incorporated in the device. In addition, this technology would allow the use other macromolecules (proteins, large peptides, mRNA) to be readily incorporated into the target cells.**

9. List all known and other possible uses for the invention. Drug, Gene, Cell, Peptide, Growth factors and other macromolecules could be delivered to various endocardial sites of the heart or other tissues. It also could be used for epicardial delivery.

10. Specifically describe the invention and its operation. You may use and attach copies of sketches, prints, photographs and illustrations which should be signed, witnessed and dated. Use numbers and descriptive names in descriptions and drawings. **See attached drawings and descriptions.**

11. List all features of the invention that are believed to be novel. **Acute macromolecule delivery using a combined catheter system with mapping capabilities, injection as well as electroporation capabilities. The fluid delivery catheter is extendable and retractable, and contains a locking mechanism for very accurate tissue injection. If a bipolar sensing signal is obtained from fluid delivery catheter tip to delivery catheter tip, a safe injection can be performed without visual control (fluoroscopy). Having such a system contained as a complete delivery system may be novel, as well as a proximal or distal screw mechanism. This technology uses a device that incorporates sensing mechanism, a delivery mechanism (needle), and an electrode to delivery an electroporation shock all in one device to allow site-specific acute localized gene delivery to the heart. In another embodiment, this technology could be used for a chronic pacemaker system as described in Medtronic patent application P10537, but also for epicardial or pericardial gene therapy.**

12. Sale or Publication (Needed to establish the date of any printed publication, public use or sale, since no U. S. patent application may be filed after one year from such date.)

    a.   If a device has been offered, or will be offered for sale, or used for profit or otherwise publicly disclosed, state when and to whom delivered and how used? _____

    b.   Has a printed description of this invention been made available to persons outside the company? How and when and was use restricted (e.g. licensing agreement, non-disclosure agreement, proprietary legends, etc.)? _____

13. Inventor(s) Signature(s) (REQUIRED):

    Daniel Sigg
    Signature _Daisy P. Cross_
    James Coles
    [signature]

Manager's Comments

14. How is this invention important to your products, plans or goals? _GENE DELIVERY IS A KEY COMPONENT OF OUR NEXT GENERATION BRADY PRODUCTS. THIS WILL BE PURSUED DURING THE TECHNOLOGY PHASE FOR THIS PRODUCT FAMILY._

15. Manager's Signature (REQUIRED)

_____   _____
Signature   Date

Manager's Printed Name *Jim Laske*   Mail Stop *B252*
Business Unit *CRM THERAPY DELIVERY*

Manager: Please forward to Patent Section of Law Department, LC340, upon completion of your review.

DOCUMENT #:	00-PR-22184XC
DOCUMENT TYPE:	Research Report
DATE:	
PROJECT #	
PREPARED BY:	Daisy Cho

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MATERIALS AND BIOSCIENCES CENTER

TITLE: Electroporation-mediated Gene Transfer: Preliminary In Vitro Studies.	**APPROVAL:** Jesus Casas-Bejar

KEYWORDS:
Electroporation, in vitro, 293 cells, monocyte, macrophage, U-937 cells, 3T3 cells, flow cytometry, anti-inflammation, IkB, gene transfection, green fluorescent protein.

Executive Summary

Transfection of cells and tissues via electroporation may prove to be an efficient method for the incorporation of genes to be used for therapeutic purposes. This report summarizes the electroporation parameters that were investigated in several cell lines and their effect on exogenous gene incorporation and expression. Under the parameters evaluated, only the 293 (human kidney fibroblast) and the U-937 (human monocyte-like) cell lines showed positive gene expression, and this was shown to be dependent on the amount of DNA added to the cell suspension and electroporation voltage. Flow cytometry analysis proved to be valuable for the quantitation of transfection efficiency in the 293 cell line. Using this analysis method, the best electroporation parameters investigated yielded positive gene expression in ~50% of the cells (293).

The results from this study may serve as the in-house basis of future progress in electroporation as a non-viral approach to facilitate gene transfer into the myocardium or other tissues.

Background Information

Electroporation is a method used to facilitate the transfer of relatively large molecules, such as plasmid DNA, into cells. This method of transfection involves the application of a high intensity electric field across cells for a short time, during which transient transmembrane pores may be formed. Plasmid DNA can gain access into the cell through these pores and may be incorporated into the host cell's genome.

Because cardiac tissue is electrically active, it may therefore be especially receptive to electroporation-mediated gene transfer.[1] It has already been reported that electroporation can occur during defibrillation.[2,3] In the presence of exogenous genes, it is our hypothesis that this phenomenon can be exploited and used towards a therapeutic advantage.

RC0068A

The combination of successful, controlled electroporation-mediated gene transfer with Medtronic's strength in defibrillation and bioelectric technology could result in a promising line of therapy for cardiac-related abnormalities.

Objectives

There were two main objectives of the in vitro electroporation project: 1) To perform and optimize electroporation-mediated transfection of primary isolated human monocytes/macrophages, and 2) To determine the cellular functionality of increasing IκB-α expression as an anti-inflammatory therapy.[1] This document summarizes the results of the experiments designed to address the first objective.

Materials and Methods

In order to gain a comprehensive understanding of the effects of electroporation, a number of cell lines were investigated.

In vitro cell culture
Five types of cells were used in the various electroporation experiments:
1) 3T3 cells (mouse kidney fibroblast) obtained from ATCC (CRL-1658)
2) 293 cells (human kidney fibroblast) obtained from Quantum Biotechnologies, Inc. (Cat No. QBI-293A)
3) Monocyte-like U-937 cells (human histiocytic lymphoma) obtained from ATCC (Cat No. CRL-1593.2)
4) Primary human monocytes isolated in our laboratory[2]
5) Human monocyte-derived macrophages (derived from primary human monocytes by culturing for 7 days)

Plasmid
EGFP-C1: pEGFP-C1 (Enhanced Green Fluorescent Protein) plasmid DNA: Scaled up from stock, Cat No. 6084-1 Clontech. Plasmid preparations were either made in-house, or were performed by ATG Laboratories (Eden Prairie, MN).

Electroporation Equipment
Electroporation equipment was obtained from Bio-Rad (Hercules, CA). The system was composed of the Gene Pulser II Module (Cat No. 165-2105) which is used in conjunction with either the RF Module (Cat No. 165-2112) or the Capacitance Extender PLUS Module (Cat No. 165-2108). Electroporation cuvettes for cells in suspension (0.2 cm gap, Cat No. 165-2086 or 0.4 cm gap, Cat No. 165-2088) were also obtained from Bio-Rad. Electroporation cuvettes made for cell culture inserts were purchased from Midwest Scientific (Cat No. ACC-001, manufactured by EquiBio, Ltd., Kent, UK).

Electroporation buffer
The choice of the electroporation buffer was dependent on the electroporation apparatus set-up. For the Gene Pulser II used in conjunction with the RF Module, PB-Sucrose was used.[3] For the Gene Pulser II, used alone or in conjunction with the Capacitance Extender PLUS, the electroporation buffer used was growth medium appropriate for the cell line (e.g., for U-937 cells, RMPI-1640 supplemented with 10% FBS).

Electroporation Protocol
The electroporation protocol for 293 cells is described in detail in Control Document 00-PR-2195/DC.[3] Briefly, adherent cells were trypsinized and placed into electroporation cuvettes. Plasmid DNA was added to the cuvettes, and the electroporation pulses were delivered with the Gene Pulser II in conjunction with the RF Module. This protocol was also followed for the electroporation of 3T3 cells.

For electroporation of cells other than 293 and 3T3 cells, some modifications to this protocol were made. U-937 cells and fresh monocytes were already in suspension and therefore do not need to be trypsinized; the rest of the electroporation protocol was followed accordingly. Monocyte-derived macrophages that were seeded onto tissue culture plates were scraped (not trypsinized) and added to Bio-Rad electroporation cuvettes for the electroporation

procedure. Inserts containing monocyte-derived macrophages were transferred to *in situ* electroporation cuvettes (EquiBio, Ltd., Kent, UK) and the rest of the electroporation protocol was followed accordingly.

Cytotoxicity Assay

The degree of cytotoxicity resulting from electroporation was quantified with the LDH Cytotoxicity Detection Kit from Boehringer Mannheim (Cat No. 1 644 793). This assay is based on the principle that cells exposed to sudden trauma may undergo rapid cell death; the resulting membrane permeability alterations allow intracellular contents to spill into the surrounding medium. Lactate dehydrogenase (LDH) is one enzyme expelled during this process.

Briefly, after electroporation, the cell supernatants are incubated with reagents from the kit. The amount of the product resulting from the enzymatic reaction is proportional to the number of dead cells, and the absorbance of this product can be measured at 490 nm.

Analysis Tools

Flow cytometry was used to quantitate the number and intensity of EGFP-transfected 293 cells.[7] Due to difficulty in analyzing U-937 cells by flow cytometry, only subjective observations (i.e., fluorescence microscopy) of transfection efficiency were made.

Results

Monocyte isolation is a somewhat time-consuming and labor-intensive procedure. As a result, preliminary experiments were carried out in well-established cell lines to become familiar with the electroporation procedure. In most of the trials, the cells were transfected with enhanced green fluorescent protein (EGFP) plasmid DNA; transfection efficiency was consequently determined by the degree of EGFP fluorescence. Below are the results of the electroporation trials performed in the different cell lines tested.

3T3 Cells

In the experiments performed in this cell line, two sets of parameters yielded positive EGFP gene expression. The first set of parameters involved the use of the Gene Pulser II, where voltage was set to 1000 V and capacitance to 1 µF; these parameters yielded a transfection efficiency of 1% (subjective observations).

In the second set of experiments, the RF Module was used in conjunction with the Gene Pulser II. In this trial, electroporation voltage was varied while all other conditions were held constant (5 bursts/4 ms interval/1 ms duration/100% modulation/25 kHz frequency). Voltages of 50 V and 100 V did not result in gene expression, whereas 200 V yielded gene expression in 1% of the cells (subjective observations). A high electroporation voltage of 400 V was very traumatic to the cells, as evidenced by a high number of unattached cells floating in the growth medium.

The transfection efficiencies were very low in this cell line, and by no means optimized. In the interest of time, we chose to focus optimization in another cell line (the 293 cell line) that had shown better results using the same preliminary electroporation parameters. Further experimentation with 3T3 cells could focus on replicating positive results from other authors.[8-10]

293 Cells

The 293 human kidney fibroblast cell line was found to be more amenable to electroporation-mediated transfection than the 3T3 cell line. As a result, the majority of the preliminary electroporation focused on using the 293 cells to investigate a number of parameters and their effects on transfection efficiency: voltage, frequency of the electric field pulses, percent voltage modulation, amount of plasmid DNA, and temperature of pre-electroporation. Flow cytometry analysis was used to quantitate EGFP expression.[7]

In this cell line, the amount of DNA and voltage appeared to be the factors most crucial in determining the degree of transfection (Figures 1 and 2, respectively). Changes in frequency, percent modulation, and incubation temperature yielded insignificant changes in the number of cells expressing EGFP.

The effect of electroporation voltage on cell viability was also investigated. Figure 2 shows the cytotoxic effects of exposing 293 cells to electroporation voltages greater than 300 V. From this figure, it can be seen that the optimal electroporation voltage range for 293 cells is 200-300 V as evidenced by the low levels of cytotoxicity and high rates of transfection.
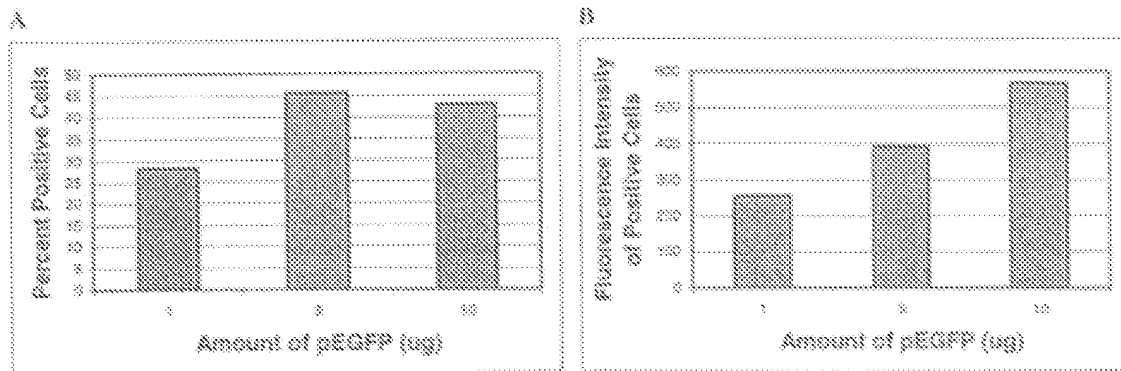
Figure 1. 293 cells subjected to electroporation in the presence of different amounts of pEGFP. Using the RF Module, electroporation parameters were set at 200V/5 bursts/4 ms interval/1 ms duration/100% modulation/25 kHz frequency, and cells were placed in a 0.2 cm gap cuvette. Transfection efficiency was assessed in terms of (A) percentage of fluorescent cells, and (B) fluorescence intensity.
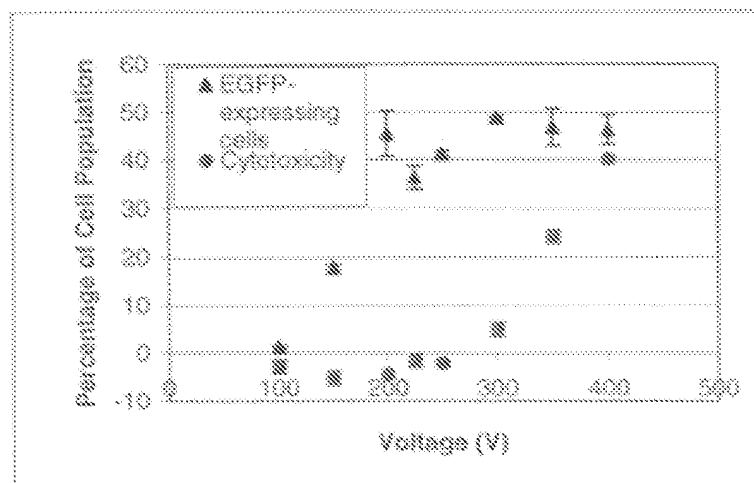

Figure 2. Effect of electroporation voltage on EGFP-C1 transfection and cytotoxicity in 293 cells. The other parameters were held constant at 5 bursts/4 ms interval/1 ms duration/100% modulation/25 kHz frequency, and cuvettes of 0.2 cm gap were used. Optimal voltage for this cell line was found to be in the range of 300-380 V as evidenced by the low levels of cytotoxicity and high rates of transfection.

U-937 Cells

As a last step before attempting monocyte transfection, electroporation trials were attempted with a human monocyte-like cell line, the U-937 cells, in an attempt to further improve the parameters for monocyte electroporation. The parameters varied in these trials included voltage, percent voltage modulation, amount of plasmid DNA, and capacitance.

In a trial using the RF Module, voltage was varied between 100-400 V, while the other electroporation parameters were held constant (5 bursts/4 ms interval/1 ms duration/100% modulation/25 kHz frequency). Results of the trial are shown in Table 1.

Table 1. Results of U-937 cells transfected with 30 μg pEGFP using the RF Module, 0.2 cm cuvette gap.

Electroporation Parameters (V/bursts/interval/dur/%mod/freq)	Number of Positive Cells (Subjective Observations)
100/5/4/1/100/25	-
200/5/4/1/100/25	+
300/5/4/1/100/25	++
350/5/4/1/100/25	++/+++
400/5/4/1/100/25	+++

Protocols provided by Bio-Rad show that the use of the Capacitance Extender PLUS module can also be used to electroporate U-937 cells.[11-13] In an attempt to enhance transfection efficiency, the use of the Capacitance Extender PLUS for electroporation was attempted, and the results of these trials are shown in Table 2. (Note: After delivering the electroporation pulse with the Capacitance Extender PLUS, the module displays the time constant and actual voltage delivered to the sample; these are listed in Table 2 under the appropriate headings.)

Table 2. Results of U-937 cells transfected with 30 μg pEGFP using the Capacitance Extender PLUS.

Electroporation Parameters	Time constant (msec)	Actual Voltage (kV)	Number of Positive Cells (Subjective Observations)
200 V, 975 μF	9.2	0.210	+
250 V, 975 μF	8.8	0.262	++
300 V, 975 μF	10.3	0.310	+++

Monocytes and Monocyte-derived Macrophages

Based on the most successful results of the electroporation attempts with U-937 cells, electroporation was performed a number of times with primary human monocytes and monocyte-derived macrophages.

Electroporation of freshly isolated human monocytes was attempted with the RF Module, with voltage settings in the range of 300-400 V (other parameters held constant: 5 bursts/4 ms interval/1 ms duration/100% modulation/25 kHz frequency, 0.2 cm cuvette gap).

Parameters stated in the literature that involved the use of the Capacitance Extender PLUS were also attempted.[14-16] These experiments involved attempts to electroporate fresh monocytes and monocyte-derived macrophages in the range of 200-300V at constant capacitance of 975 μF.

The last electroporation trial was performed to eliminate any adverse effects of scraping the cultured monocytes from the plates. Monocytes were allowed to grow in tissue culture inserts for 7 days, and the monocyte-derived macrophages were electroporated with the Capacitance Extender PLUS in cuvettes specially designed for inserts (EquiBio, Ltd).

Unfortunately, the above experiments were unsuccessful and did not facilitate pEGFP gene expression by monocytes or monocyte-derived macrophages.

Summary

A number of cell types were studied regarding their ability to be transfected via electroporation. Our main goal in this phase of the project was to determine optimal electroporation parameters using the EGFP marker gene. Once the parameters were defined, the next step was to apply these results towards the introduction of an anti-inflammatory gene.

Successful monocyte transfection is very difficult, as evidenced by a number of publications.[14-17] At best, published protocols showed transfection efficiencies in the order of 1 in 500 cells.[15] These results may have been achieved in our trials, but may have been overlooked as background. Indeed, transfection efficiency would have to be increased if gene incorporation into monocytes is to be of any therapeutic value. Because our attempts to transfect these cells were thus far unsuccessful, further electroporation studies of monocytes/macrophages will be placed on hold to await more promising results from academic laboratories.

Results obtained from electroporation experiments with the 293 cells, on the other hand, are promising and show that electroporation is a realistic method for facilitating gene transfer. This method may be applicable for use in

ex vivo transfections for therapeutic purposes (i.e., cell transplants). Additional fibroblast electroporation experiments may be performed since these cells are involved in inflammation-associated fibrous capsule formation in response to implanted devices. Further work involving the IkB-α gene will likely be done *in vivo* since the anti-inflammatory effects of this gene have recently been proven *in vitro*.[18]

References

1. Harrison RL, Byrne BJ, and Tung L. Electroporation-mediated gene transfer in cardiac tissue. FEBS Letters 435: 1-5 (1998).
2. Tovar O and Tung L. Electroporation and recovery of cardiac cell membrane with rectangular voltage pulses. Am. J. Physiol. 263: H1128-H1136 (1992).
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17. Simões S, Slepushkin V, Pretzer E, Dazin P, Gaspar R, Pedroso de Lima MC, and Düzgüneş N. Transfection of human macrophages by lipoplexes via the combined use of tranferrin and pH-sensitive peptides. The Journal of Leukocyte Biology 65: 270-279 (1999).
18. Griesenbach U, Scheid P, Hillery E, de Martin R, Huang L, Geddes DM, and Alton EWFW. Anti-inflammatory gene therapy directed at the airway epithelium. Gene Therapy 7: 306-313 (2000).

Medtronic Laboratory Notebooks 9912, 9964, 10060.

Electroporation of Chick Heart
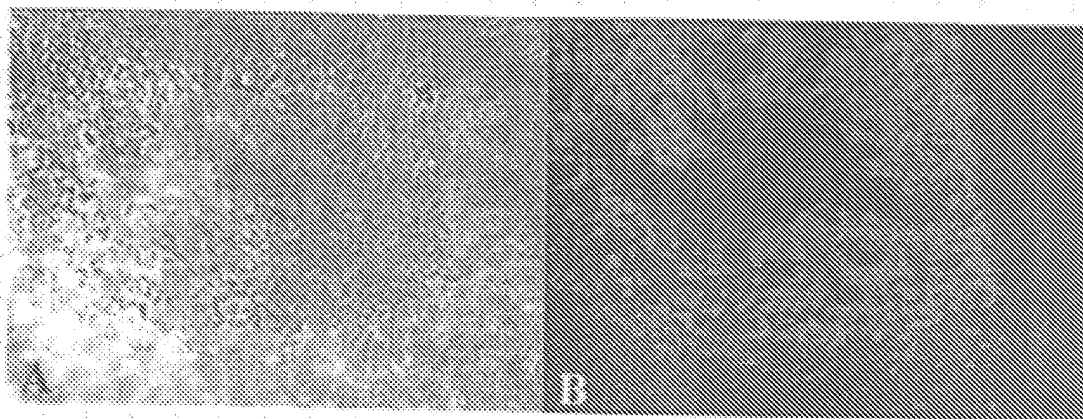## with pEGFP-C1 Plasmid DNA
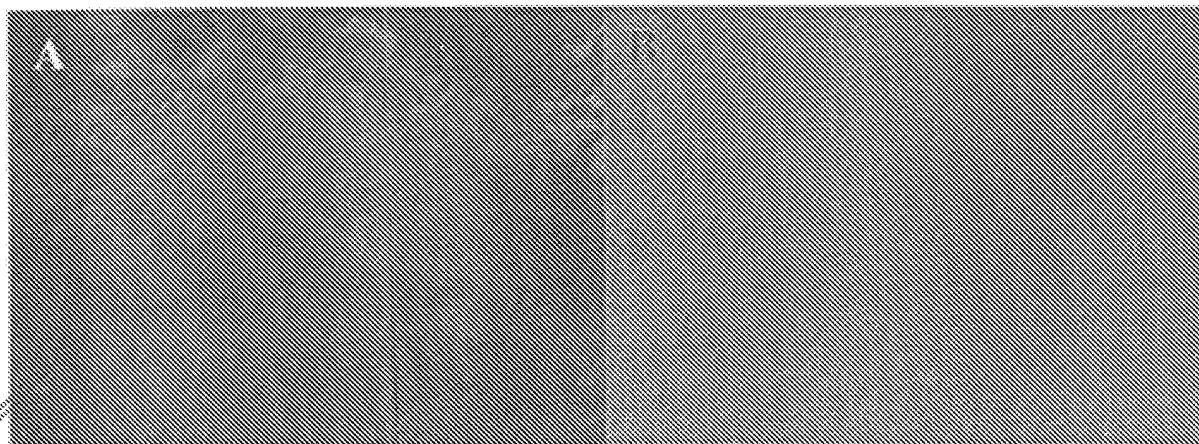
(A, GFP fluorescence image at 20X magnification; B, Bright field
image at 20X magnification; Tissue exposed to 1x400V, 1x350V,
2x380V triplet bursts)

Results (5-10-99)
- analyzed electroporation of chick heart
- surface of tissues appeared to take up ??? & flourescent ???
    - froze down ??? in OCT w/ liquid nitrogen ??? frozen sections

Flourescent images of frozen section of
chick embryo heart (?) - ??? show
bright field images.

Conclusion: Electroporation of chick embryo heart is possible. It
appears that with the electroporation parameters used, ???
penetration of the ??? in ??? ??? It would be interesting
to see whether isolation of cardiac myocytes is possible
after electroporation.

_o No. _____

_____ : to evaluate electroporation of chick embryo heart

_thod:

- Chick hearts at E11
- Electroporator parameters:
  Voltage 400V
  % Modulation = 0
  KHz frequency = 50
  burst duration = 10 usec   } Electro myfect
  # Bursts = 3
  burst interval = 10 s

  → # shock products = 0.5
  → Channel Buffer = Spry/mL, pAV 400 – Fugal

  → Used another in situ electroporation system
    - filled lower chamber w/ 40mL Betaine media
    → filled cell insert w/1.5 ml pDS/Betaine buffer
      50 µg/ml DNA

    - assay 11-15-99 (M) (100 µg/ml chick)
      - not fixed in 4% Los cucore

  A ⓐ 1 × 400 V
  B ⓑ 2 × 300 V
  C ⓒ 1 × 400 V, 1 × 350 V, 2 × 300 V
  D ⓓ 1 × 400 V, 1 × 330 V, 2 × 200 V
  E ⓔ 1 × 400 V, 1 × 350 V, 1 × 200 V
  F ⓕ control (no ... buffer, no modulation